Heliyon 7 (2021) e07721

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Bioactivity evaluation for volatiles and water extract of commercialized star anise

Bassem Ahmed Sabry [a], Amr Farouk [b], Ahmed Noah Badr [a,*]

[a] Food Toxicology and Contaminants Dept., National Research Centre, Dokki 12622, Cairo, Egypt
[b] Chemistry of Flavor and Aroma Dept., National Research Centre, Dokki 12622, Cairo, Egypt

ARTICLE INFO

Keywords:
Commercial star anise
Essential oil
Water extract
Cytotoxicity
Anti-mycotoxigenic
Antimicrobial

ABSTRACT

Background: Usually, it takes about a year since the harvested fresh star anise fruit (SAF) reaches the market for consumer usage, all this time with different handling processes and different storage circumstances greatly affect its quality as well as its chemical composition and biological activity.
Aim: This study investigated the chemical constituents for volatiles and water extracts of commercialized SAF, as well as, their bioactivities.
Results: The chemical constituents were dominated by Trans-*Anethole* (47.16 %), estragole (14.4 %), and foeniculin (8.86 %) in the essential oils. Meanwhile, Coumarin, Apigenin, and Rosmarinic were the predominant phenolics of water extract. The result reflects a distinction of water extract to minimize mycotoxin secretion in liquid media. The SAF-volatiles were more effective in inhibiting microbial growth of the investigated bacterial and fungal strains.
Conclusion: Although samples were commercially collected from markets, their extracts were still capable to inhibit up to 55 % of fungal growth. The SAF water extract exhibited a moderate and selective cytotoxic effect (IC_{50} = 114.9 μg/ml) against HepG2 cell lines compared to the low impact of essential oil (IC_{50} = 513.8 μg/ml). Which led to the conclusion that despite the long-time span for SAF till it reaches the market, aqueous extract maintained a good ability for reducing mycotoxins-secretion from fungi grown in liquid media. This result emphasizes the role of the phenolics of water extracts' as an anti-mycotoxigenic agent.

1. Introduction

The dried fruits of *Illicium verum* Hook. F., which is also known as Chinese star anise and cultivated in the Southeast of China and North of Vietnam, represents one of the most important among the species of the *Illicium* genus [1]. The star anise fruits (SAF), as well their volatiles and extracts, are utilized for flavoring foods, bakeries, drinks, and candies, in addition to their utility in traditional medicine as a stimulant, carminative, stomachic, and galactagogic [2]. Medicinal plants are often stored for long periods before being used as raw materials for manufacturing, which could happen due to the conditions of importing, clearance, distribution, or others. The storage period indicated for medicinal plants in markets is approximately up to one-year. More extended periods of validity might be accepted if the manufacturer presents stability tests, which prove the maintenance of the product's characteristics during the proposed period [3].

A Significant factor that may affect the evaluation of biological activity was the extraction technique. The efficiency of extraction is based on the extraction method, extraction solvent, and extraction time. The desired compounds extracted influence the choice of extraction and the extraction conditions [4], where Onyebuchi, and Kavaz, reported that temperature and solvent type affect the bioactive potential of *Ocimum gratissimum* L. extracts [5]. Variation in extraction techniques will lead to the modulation of extracted component types and quantities. These extracted components are the ones that will perform the bioactivity impact against microbial hazards including toxigenic fungi and mycotoxins.

Improper storage can result in physical, chemical, and microbiological changes, besides exposure to contamination, particularly by toxigenic fungi that secrete mycotoxin. Toxigenic fungi are known to represent a health hazard to humans, and responsible for food loss issues [6]. Mycotoxins, particularly aflatoxins, represent a public health concern for food-based material production. It often contaminates the agricultural

* Corresponding author.
E-mail address: noohbadr@gmail.com (A.N. Badr).

https://doi.org/10.1016/j.heliyon.2021.e07721
Received 10 May 2021; Received in revised form 17 June 2021; Accepted 2 August 2021

products in both the field and the stored ones [6, 7]. Regarding the previous studies, an anti-aflatoxigenic impact of the SAF was reported for the volatile oils, but there is no referring for the water extract's impact [8]. Moreover, its impact on other mycotoxins like zearalenone and ochratoxins was not assessed properly yet. The elucidation of how both the SAF-volatile oils and water extract can limit the hazard of mycotoxigenic and fungal contamination is still needed for more discussion.

Various articles reported the biological activities of the SAF volatile oils focusing on their antimicrobial, antioxidant, anti-mycotoxin activity [8, 9]. However, as far as, the authors' knowledge, the cytotoxic or the anti-proliferative activities of SAF volatile oils or extracts have not been reported yet. Most of the published studies have dealt with the authenticated fresh type of the SAF, but not the commercial or actual-marketed one, which did not represent clear data about the actual consumed type. Seizures and other neurological effects have been reported due to adulterated Chinese star anise fruits with other similar toxic species, especially Japanese star anise (*I. anisatum*) [10] and that raised a genuine concern about the marketed commercialized star anise.

Therefore, the study aimed to investigate the bioactivity and the safety of the actual marketed SAF type, and this was achieved by evaluating volatile oil composition and water extract. The evaluation was also inclusive of antimicrobial and anti-mycotoxigenic activities, as well as, the cytotoxic impacts on liver cell-line tissues in comparison between volatile oil and water extraction techniques. Because the SAF is applied in drinks, traditional medicine, and food-spice to achieve their distinctive properties, the results of the present investigation can provide a guide for the utilization of commercialized star anise fruits in food processing, it may also clarify their performance efficacy to limit fungal contamination and mycotoxin secretion.

2. Materials and methods

2.1. Plant material, chemicals, cell lines, and microbial strains

The SAFs of Vietnamese origin were purchased from a reliable herbal shop, Dokki, Cairo, Egypt. The phenolic standards were purchased from Sigma Chemical Company (St Louis, MO, USA). The mixture of n-alkanes (C_6–C_{26}), authentic compounds, sodium sulfate anhydrous, cisplatin (CIS), and tetrazolium bromide solution (MTT) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

Human hepatocellular carcinoma (HepG2) and normal liver (THLE2) cells were purchased from the VACSERA (Cairo, Egypt). DMSO was provided by Merck, Darmstadt, Germany. Fetal calf serum (FCS) and penicillin/streptomycin were obtained from Hyclone, Logan, UT, USA. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA.

Applied strains of microorganisms for antimicrobial evaluation were 4 bacterial and 4 fungal strains. Bacterial strains were identified species of food-pathogenic bacteria, including *Escherichia coli* ATCC 11228, *Bacillus cereus* ATCC 11778, *Salmonella typhi ATCC 14028*, and *Staphylococcus aureus* ATCC 33591. Utilized fungal strains were *A. flavus* ITEM 698, *Aspergillus ochraceous* ITEM 282, *A. carbonarius* ITEM 5011, *Fusarium oxysporum* ITEM 12591, *F. culmorum* KF 191, *F. moniliforme* KF 488, and *Penicillium chrysogenum* ATCC 10106.

Table 1. The phenolic compounds content of SAF water extract.

Compound	R_t (min)	Conc. (μg/g extract)
Gallic	3.97	27.3 ± 1.33
Protocatechuic	7.35	344.5 ± 4.58
p-hydroxybenzoic	11.29	32.9 ± 1.12
Gentisic	12.29	10.8 ± 1.05
Cateachin	14.64	22.2 ± 1.34
Chlorogenic	15.95	9.3 ± 0.88
Caffeic	16.22	13.15 ± 1.02
Syringic	18.15	40.08 ± 1.41
Vanillic	21	12.78 ± 1.27
Salycilic	24.88	79.77 ± 2.15
Ferulic	26.47	56.45 ± 2.37
Sinapic	30.156	4.85 ± 0.66
p-coumaric	34.4	48.00 ± 1.05
Rutin	33.98	75.18 ± 1.73
Apigenin-7-glucoside	37.93	299.39 ± 5.27
Rosmarinic	38.8	415.89 ± 4.97
Cinnamic	39.3	43.22 ± 1.56
Quercetin	45.3	130.50 ± 2.05
Coumarin	55.22	275.67 ± 2.77
Kaempferol	58.4	70.99 ± 1.33
Chrysin	58.44	68.76 ± 1.58

The data expressed as mean ± SEM.
Concentrations were calculated in micrograms per gram of lyophilized extract.

2.2. Preparation of the water extract

Purchased fruits of the SAF were air-cleaned, dried, then it was extracted using a pressed warm-water (37°C/18h) as described by Abdel-Salam *et al.* [11]. The collected extract was sterilized using a sterile membrane (0.22 μm), and lyophilized by a Dura-Dry MP freeze-dryer (FTS System, USA).

2.3. Extraction of the volatile oil

Extraction of the volatile oil from the SAF was carried out by hydro-distillation (3 h), using a Clevenger type apparatus. The extracted essential oil was dried using anhydrous sodium sulfate, stored in airtight glass vials covered with aluminum foil at (−20 °C) until analysis [12].

2.4. Phenolic compounds determination using HPLC

The phenolic content of water extracts was determined as the same methodology described by Badr et al., [13]. The analysis was performed using an Acquity H class UPLC system equipped with a Waters Acquity PDA detector (Waters, USA). The condition and the column characteristics were the same as described before.

2.5. Volatile content determination using the GC–MS

Analysis of volatile constituents from the hydro-distilled (HD) oil was carried out using the GC–MS apparatus. A Trace GC Ultra Chromatography system equipped with an IQS-mass spectrometer and a 60 m × 0.25 mm × 0.25 μm-thick TG-5MS capillary column (Thermo Scientific, USA) was programmed from 50 °C with a holding time of 3 min. Then the temperature was increased at a rate of 4 °C per min to 140 °C with a holding time of 5 min. After that, the temperature was increased at 6 °C per minute to 260 °C for a 5-min isothermal holding time. The injector temperature was 180 °C, the ion source temperature was 200 °C, and the transition line temperature was 250 °C. The carrier gas was helium with a constant flow rate of 1.0 mL/min.

The mass spectrometer had a scan range from m/z 40–450, and the ionization energy was set at 70 Ev. The identification of compounds was based on matching with the MS computer library (NIST library, 2005 version) and comparing authentic compounds and published data [8, 14, 15]. The relative percentage of the identified constituents was calculated from the GC peak areas. The Kovats index was calculated for each compound, using the retention times of a homologous series of C_6–C_{26} n-alkanes and by matching with the literature [14].

2.6. Antibacterial and antifungal activity determination

The antimicrobial activity of the SAF volatile oil was performed using the agar well diffusion method [16]. It was evaluated against 4 bacterial and 4 fungal strains. One hundred microliters of the inoculum (100μL contains 1×10^9 CFU/mL for bacteria, or 100μL contains 2.6×10^6 CFU/mL for fungi) were mixed with specific media of each microorganism and poured into Petri-plates. The test compound was introduced into the formed well in each Petri dish (100μL/5 mm). Plates were incubated (37°C/36 h) for bacterial strains, and (28 °C/96 h) for fungi. The diameter (mm) of the resulted zone-inhibition was measured.

2.7. Antimicrobial activity measuring assay

The minimal antibacterial concentration (MIC) was determined by a micro-dilution method using serially diluted extracts according to the NCCLS protocol [17]. The volatile oil and water extract were diluted to get a series of concentrations from 5-500 μL/mL in sterile broth media. The media (350μL) were spotted in microarray wells to be ready later for investigation by Eliza technique. The microorganism suspension of 50μL was added to the broth dilutions. These were incubated (18 h/37 °C). The MIC value was recorded as the lowest concentration that did not give any visible bacterial growth.

The fungicidal effect was assessed by mycelia growth inhibition of utilized fungi and recorded by the Eliza spectroscopic analysis at 595nm wavelengths [18]. The activity was assayed using Czapek-Dox broth media contain the spore solution (96 h incubation/28 °C). A volume of fungal spore suspension (10uL contains 10^5spores/mL) was injected for each well of a micro-array plate. Nystatin was used as a standard antifungal control. The MFC was seen in the non-fungal growth on the microarray plate.

2.8. Determination of extracts-impact on toxigenic producing fungi

Using a medium of the Czapek-Dox broth, containing the spore solution of *Aspergillus flavus* ITEM 698 (1×10^5 spores/mL), the impact of the SAF on the aflatoxins production was evaluated as the same methodology described by Shehata *et al.*, [19]. While the effect on Zearalenone production determined in a medium contains *F. culmorum* KF191 spores. Serial dilutions of the SAE (0, 5, 10, 15, and 20 mg/mL) in 50 mL broth media were prepared in 250 mL conical flasks. These flasks were inoculated by a spore suspension of each fungus and were shaking incubated (28 °C/200 RPM/7 days). The fungal dry weight and mycotoxin production were determined using the same conditions described in published methods [19].

The culture broth was filtered using filter paper (Whatman No.1). The fungal residue was washed with sterile water and then allowed to dry at 80 °C until a constant weight was obtained. The aflatoxin content in the culture supernatant was determined by the cleanup step for mycotoxin production done using the immune affinity columns; Aflatest® for Aflatoxins Zearatest® for Zearalenone. Columns were washed three times, eluted to vials, drying to form a film, and finally kept until HPLC evaluation. The mycotoxins content in the cultured supernatant was determined using HPLC (Waters Corporation, USA) equipped with a multi-wavelength fluorescence detector and a Phenomenex RP-C_{18} (250 mm × 4.6 mm id, 5 μm) reverse phase column.

Table 2. Volatile constituents identified from the *star anise fruits* hydro distilled (HD).

No.	Identified Compound	KI[a]	% Area[b]	Identification method[c,d]
1	*α*-Thujene	928	0.43	MS & KI
2	*α*-Pinene	932	**2.48**	MS, KI, & ST
3	Camphene	971	0.44	MS & KI
4	*β* – Pinene	978	0.28	MS & KI
5	*β* –Myrcene	991	0.27	MS & KI
6	Linalool	1095	**1.92**	MS, KI, & ST
7	Estragole	1194	**14.4**	MS, KI, & ST
8	*cis*- Anethole	1250	**2.26**	MS, KI, & ST
9	Linalool acetate	1254	**1.96**	MS, KI, & ST
10	*trans* – Anethole	1281	**47.16**	MS, KI, & ST
11	Safrole	1292	0.21	MS, KI, & ST
12	*α*- Copaene	1373	0.37	MS & KI
13	*β* –Caryophyllene	1414	**1.24**	MS, KI, & ST
14	*trans*- *α*- Bergamotene	1433	**1.42**	MS, KI, & ST
15	Aromadendrene	1436	**1.68**	MS, KI, & ST
16	*trans*- *α*- Bergamotene	1465	0.33	MS & KI
17	*β* – Bisabolene	1502	**1.12**	MS, KI, & ST
18	*μ*- Cadinene	1510	0.70	MS & KI
19	Elemol	1544	0.49	MS & KI
20	Germacrene B	1553	0.91	MS & KI
21	*trans* – Nerolidol	1560	**1.01**	MS, KI, & ST
22	Spathulenol	1572	0.27	MS & KI
23	Caryophyllene oxide	1577	0.23	MS & KI
24	*α*- Cadinol	1649	0.55	MS & KI
25	Foeniculin	1672	**8.86**	MS, KI, & ST
26	Farnesol	1692	0.50	MS & KI
- -	**Total**	- - - -	**91.49**	- - - - -

[a] Confirmed by comparison with Kovat's index on a DB5 column (Adams 2007).
[b] Values represent averages ±standard deviations for triplicate experiments.
[c] Confirmed by comparison with the mass spectrum of the authentic compound.
[d] Identification by comparison with data obtained from the NIST mass spectra library.

2.9. Evaluation for SAF volatile oil and water extract cytotoxicity using MTT assay

Both Hep G2 and THLE2 were cultured at a density of 1×10^4 cell/well (100µl) in a culture medium (DMEM) supplemented with antibiotics (10,000 penicillin unit and 10 mg streptomycin in 0.9 % saline) and 10 % serum (FBS), incubated (24 h/37°C/5 % CO_2). After cell attachment, quantities of 1000 to 31.25µg SAF were applied to each cell line of THLE2 and HepG2 cells.

For water extract, serially diluted samples were used with concentrations of 3.125–100 µg/ml for HepG2 cells and 31.25–1000 µg/ml for THLE2 cells. Positive control (CIS) at concentrations ranging from 200 to 6.25 µg/mL for THLE2 and from 100 to 3.125 for HepG2 cells were applied in parallel. Subsequently, 10µl of 12 mM MTT stock solution (5 mg/ml MTT in sterile PBS) was added to each well.

After incubation for 4 h at 37 °C, the MTT solution was eliminated, and the precipitated purple formazan crystal was dissolved in DMSO for 20 min. Negative control of 10µl of MTT stock solution was added to 100µL of the uncultured medium. With an ELISA reader, absorbance was measured at 570nm. The curve was illustrated based on a variety of proportions of surviving cells according to concentrations, and IC_{50} was calculated using the sigmoidal curve obtained using Eq. (1) (Equ. 1) [20] as follow:

$$IC_{50} = [(OD_S - OD_B) / (OD_C - OD_B) \times 100\%] \quad (1)$$

OD_S: optical density of the sample,
OD_C: optical density of the control,
OD_B: optical density of the blank.

2.10. Statistical analysis

The results were statistically analyzed using the SPSS software version 16. The analysis of variance (ANOA one-way) test was used to evaluate the data, which were expressed as mean ± SD. All tests were performed in triplicate (n = 3).

3. Results and discussions

3.1. HPLC analysis of the SAF water-extract

The data represented in Table 1 shows the phenolic compound profile of the SAF water extract. Among 21 phenolic compounds detected, Rosmarinic, Protocatechuic, and Coumarin were the dominants. Within the phenolic compounds, flavonoids were identified in the analyzed extract as Apigenin derivatives, Quercetin, and Rutin at 299.39, 130.5, and 75.18 µg/g of extract, respectively. Both flavonoids and phenolic components presented widely in herbal medicines have been reported due to their efficiency as antioxidants, anticancer, antibacterial, cardioprotective, anti-inflammation, and immune system promoting skin protection from UV radiation and anti-mycotoxins agents [21].

The phenolic compounds could be divided into two major sections phenolic acids and flavonoids. In previous studies, the phenolic acid compounds showed variation both in composition and concentrations, while Aly *et al.* [8], referred to the phenolic acid content to be Ferulic, Catechin, Gallic, Caffeic, Cinnamic acids; while the flavonoid content was referenced as Rutin. Notwithstanding, Zidan *et al.* [22], identified 19 phenolic acids and referred to the derivatives of Apigenin, Quercetin, and Kaempferol as major flavonoids.

The biological activities are regularly joined to the volatile oil; the authors tend to support the existence of complementary functions between phenolic and volatile oil to express the SFA-biological activity, and even further they adopt the theory that nominates phenolic components to be the effective agent responsible for antifungal activity. The impact can be attributed mainly to phenolics whose effect is supported by antimicrobial volatiles including *trans*-Anethole and Estragole, as well as, Foeniculin [23].

3.2. GC-MS analysis of the SAF volatile oil

Twenty–six compounds were identified in the volatile oil of the traded *I. Verum* under investigation, representing 91.28 % of the total oil (Table 2). The *Trans*–Anethole (47.16 %), Estragole (14.4 %), and Foeniculin (8.86 %) were the dominants in the volatile oil, followed by α-Pinene (2.48 %), *cis*-Anethole (2.26 %), Linalool acetate (1.96 %), and Linalool (1.92 %). Some quantitative differences could be observed compared to previously published studies dealing with oil extracted from freshly collected fruits, whereas *trans*-Anethole was the predominant constituent with ≥80 % [8, 9, 24]. However, the present study findings are consistent with Howes *et al.* [15], who studied the volatile constituents of stored and traded star anise fruits, they were reported *trans*--Anethole with 5.1–29.7 % and 36.5–81.1 % for different fruit samples collected from the UK customs offices and Chinese Medicinal Plants Authentication Centre (Royal Botanic Gardens, Kew). Therefore, in this plant type, the fruits and their volatiles' quality seem to be negatively affected by the plant's storage during import and transit from a place to another and the conditions of transportation and storage.

The volatile profile of the oil extracted from the SAF under the present study is characterized by the higher content of *trans*-Anethole and Foeniculin, which indicates the absence of adulteration with the fruits of *I. anisatum* (Table 2). Additionally, *I. anisatum* volatile oil markers, e.g., Eugenol, Methyl Eugenol, Safrole, and Myristicin that were detected previously [25] were not detected in the present investigation. However, other species of star anise such as *I. anisatum*, *I. lanceolatum*, *I. brevystylum, I. henryi, I. majus,* and *I. simonsii,* were reported to be toxic and to induce dizziness, nausea, vomiting, and convulsions upon ingestion [26].

So, it is crucial to distinguish among different species using fast and reliable techniques, e.g., GC-MS, to control and detect adulteration. Elimicin was reported as a characteristic compound of the *I. lanceolatum* volatile oil and could be identified in *I. griffithii* oil [26, 27], but absent in all other *Illicium* species, which is agreed with our results (Table 2). Meanwhile, Limonene, which is absent in the current study, constitutes a dominant component among the volatiles identified in many other *Illicium* species and could be used as a marker for *I. brevistylum* (1.2–11.1 %); *I. henryi* (12.4–32.8 %); *I. majus* (3.5–10.2 %); and *I. micranthum* (2.9–17.9 %) [15].

Although the content of *trans*-Anethole in the present study was recorded with a low content comparing to numerous studies [8, 9, 28], there are still investigations were reported *trans*-Anethole by a ratio less than 90 % of the volatile oil of the SAF [14, 22, 29]. The present results of volatile oil recorded valuable quantities of Estragole, Foeniculin, α-Pinene, and Linalool fractions. These compounds were classified with antimicrobial and antifungal properties [23, 30].

3.3. Evaluation of the biological activity

In light of the results shown in Figure 1, the water extract and volatiles of the SAF manifested efficiency against the tested bacterial strains, yeast, and fungi. It was noted that; the volatile compounds were more efficacious against the bacterial strains where the results have shown significantly different, also it was more significantly inhibit fungal growth of applied strains. The pathogenic strains of bacteria were more resistant, particularly the gram-negative strains. This result could be attributed to the different cell membrane structure of gram-negative strains, where their cell wall is classified to be largely impermeable [31].

Moreover, the data gave a piece of evidence that the volatile oils' demonstrated higher antibacterial efficacy than water extract against four strains of food-pathogen bacteria. The extracts' impact was manifested by significantly different for gram-positive (G+) bacteria than the gram-negative (G-) ones. *B. cereus* ATCC 11778 was the most affected strain, while *S. typhi* ATCC 14028 was the least affected strain of the

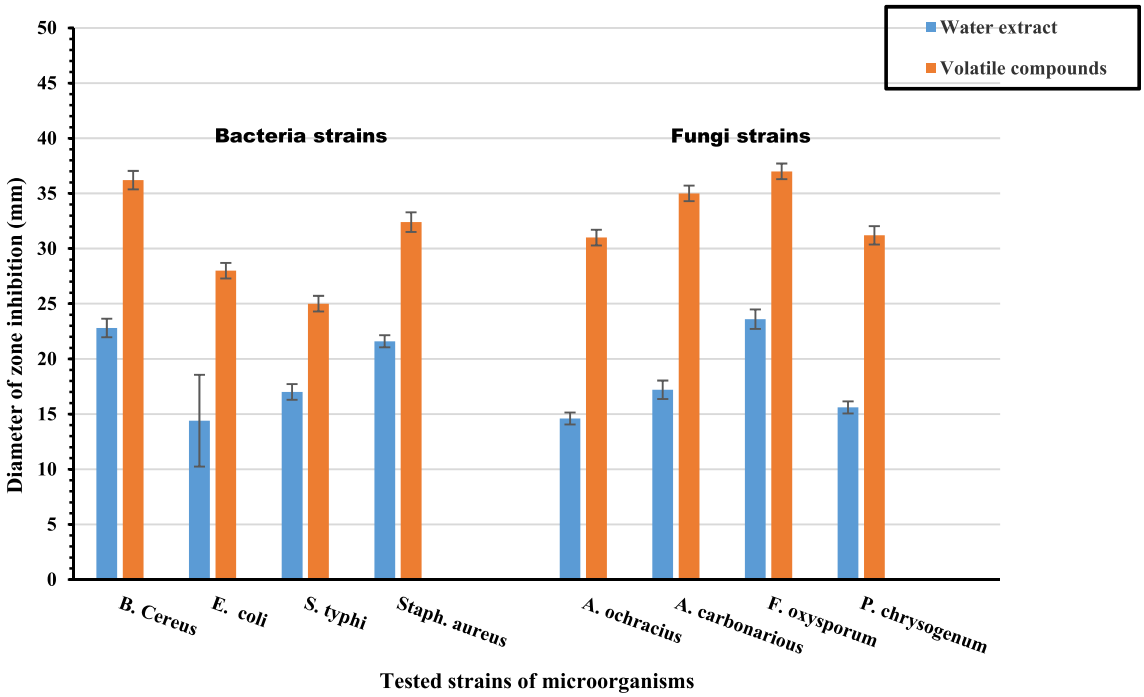

Figure 1. Antimicrobial activity of the SAF (water and volatile extracts) against pathogenic bacteria and toxigenic fungi.

tested bacteria. Again, the (G-) bacteria's resistance to the extract effect could be related to the impermeable characteristics of their cell wall.

3.4. Antifungal effect of the SAF water and volatile extracts

The antifungal diffusion assay of the two types of extracts was determined against four strains of toxigenic-producing fungi (Figure 2). A toxin-type production distinguished each strain of them. The assay result here revealed that the extracts' impact on the fungal growth, was reflected also in their ability and amount of the produced toxin. *A. carbonarius* strain was the most resistant strain followed by *A. ochraceus*, where both of them could produce ochratoxin in the growth media. The notable finding in these results joined to *F. culmorum,* a zearalenone producing strain that deemed the less resist the strain of tested fungi.

The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of the SAF extracts were determined in the broth media. The water extract results reflected that; MIC values for applied bacteria strains were ranged between 16.4 ± 1.34 to 29.6 ± 1.69 μL SAF/mL of media broth. The MFC recorded values ranged between 52.6 ± 1.82 to 73.4 ± 1.52 μL SAF/mL of media broth. Otherwise, for volatile oil extract, MIC values for applied bacteria strains were ranged

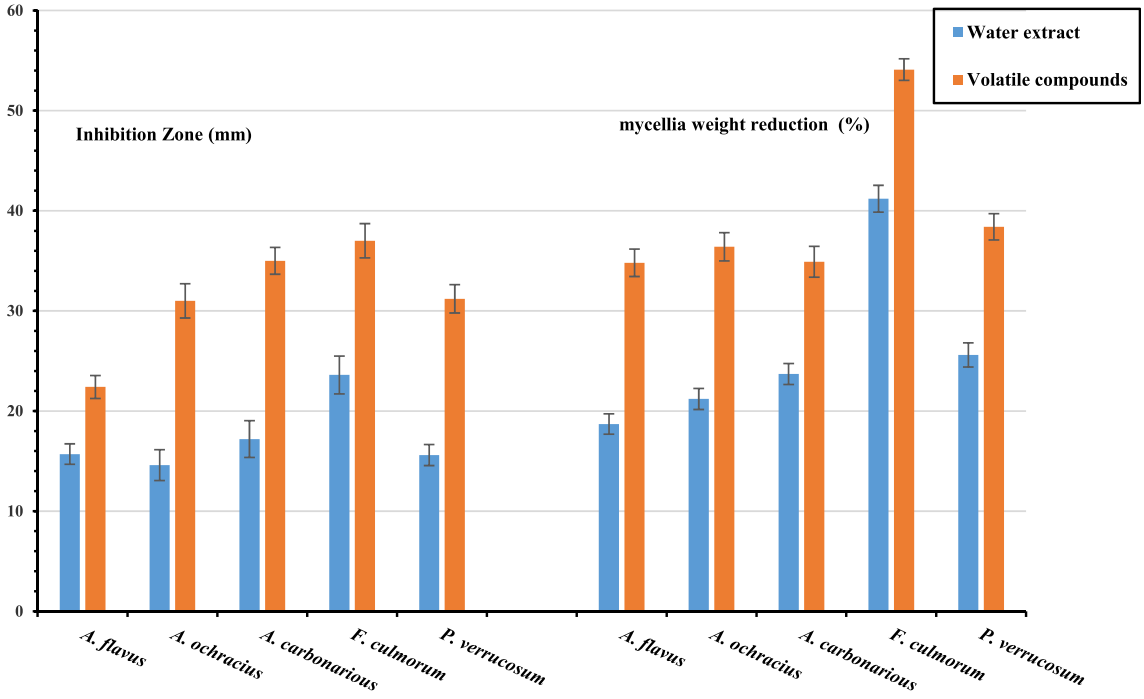

Figure 2. The inhibition impacts the SAF extracts on fungal growth (agar diffusion and liquid media).

Table 3. The impact of the SAF extracts on mycotoxin production of fungi growth in liquid media*.

	Control**	100 μL VE/mL media***	Inhibition Ratio	100 μL WE/mL****	Inhibition Ratio
AFB_1 (ng/L)	349.2 ± 1.3^a	125.7 ± 2.34^b	64 %	85.24 ± 2.09^c	75.58 %
AFB_2 (ng/L)	119.4 ± 1.34^a	92.0 ± 2.05^b	22.95 %	39.8 ± 1.36^c	66.6 %
AFG_1 (ng/L)	119.6 ± 1.67^a	85 ± 2.88^b	28.93 %	33.14 ± 1.04^c	72.41 %
AFG_2 (ng/L)	119.4 ± 3.41^a	87.4 ± 4.11^b	26.8 %	26.4 ± 2.54^c	77.89 %
ZEN *(μg/L)*	452.6 ± 8.96^a	186.2 ± 4.31^b	61.08 %	74.7 ± 6.05^c	83.49 %

The results expressed as means ± SEM.
Results with a different superscription letter are significantly different (P = 0.05).
The control measured as toxin secreted by fungi in control growth media.
VE: volatile oil extract; WE: water extract.
* mycotoxin production was estimated for Aflatoxins (AFB1, AFB2, AFG1, and AFG2) and zeralenone.
** Control express the toxin amount secreted in fungal media without the treatment.
*** Toxin amount determined in fyngal media contains 100μL volatile extract of star anise /mL of growth media.
**** Toxin amount determined in fyngal media contains 100μL water extract of star anise /mL of growth media.

between 6.6 ± 0.55 to 10.0 ± 1.0 μL SAF/mL of media broth. The MFC recorded values ranged between 133.8 ± 2.84 to 178.8 ± 8.17 μL SAF/mL of media broth.

This result points out that; the extract concentration needed for the bacterial inhibition was less in the case of using the volatile oil. These results are related to the compounds identified using GC-MS analysis. A robust antibacterial effect was recorded here for the volatiles of star anise due to the presence of linalool acetate and farnesol, which were previously reported with antibacterial effects [32, 33]. The results may spotlight its future application as an antibacterial agent in food manufacturing. Regarding the antifungal impact, the result was clearly implied more efficacy of water extract. This result could be explained through phenolic compounds' content, which is presently more in the polar extraction [34].

3.5. The inhibition impact of the SAF extracts on fungal growth in liquid media

Two strains of fungi, *A. flavus* ITEM 968 and *Fusarium colmorum* KF191 were chosen for the mycotoxin reduction assay. These strains could produce aflatoxins and zearalenone, respectively. Table 3 showed the efficacy of water extract to suppress toxin production's fungal ability even more than the volatile oil. This could be related to the differentiation in compounds identified in water extract and volatile ones. The phenolic compound in the water extract supports the anti-mycotoxigenic effect more than the volatile oil [35, 36, 37]. At a concentration of 500μL, the highest suppression of toxin production was recorded for the two extracts. At this concentration, the strain *F. culmorum* KF191 loses its ability growth on the media. In the same regard, numerous studies were referred to the antifungal impact of the SAF extracts, particularly the toxigenic fungi [8, 9, 28, 38].

3.6. Impact of the SAF water and volatile extracts on mycotoxin production

For a media used in *A. parasiticus* growth, aflatoxins levels were determined. Zearalenone toxin was also determined in the medium applied for *F. culmorum* growth, (known to produce ZEN). The inhibition ratio of toxin production increased by increasing the extract concentration in fungi' medial growth. At a concentration of 150μL water extract/mL media, there was no detectable zearalenone in the tested media, while at 200μL water extract/mL media, aflatoxins and zearalenone were not detected in the two extracted media.

3.7. In vitro cytotoxic activity of the SAF volatiles and water-extract on Hep G-2 and THLE2 cell lines

The MTT viability assay was used to investigate the in vitro cytotoxic activity of the SAF volatile oil and water extract on Hep G-2 and THLE2 cell lines compared to CIS as a reference drug (Figures 3 and 4). The cytotoxicity impact of water and volatile extracts of star anise were calculated according to the Equ.1 as explained before. The SAF water extract showed a higher cytotoxic effect (IC_{50} 114.9 μg/mL) than the volatile oil (IC_{50} 513.8 μg/mL). The reduction in cell viability percentage of the liver cancer cell line (Hep G-2) after treatment with SAF-extracts compared to treated THLE2 cells indicated the selectivity of the

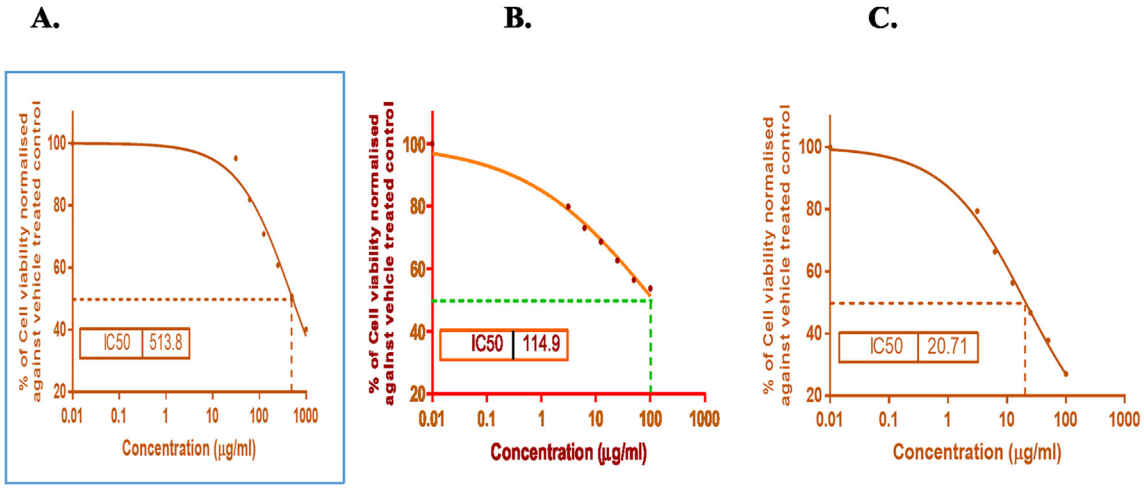

Figure 3. Evaluation of cell viability percentage of the liver cancer cell line (Hep G2) post-treatment A- SAF volatile compounds, B- SAF water extract compared with reference drug C– CIS using MTT assay.

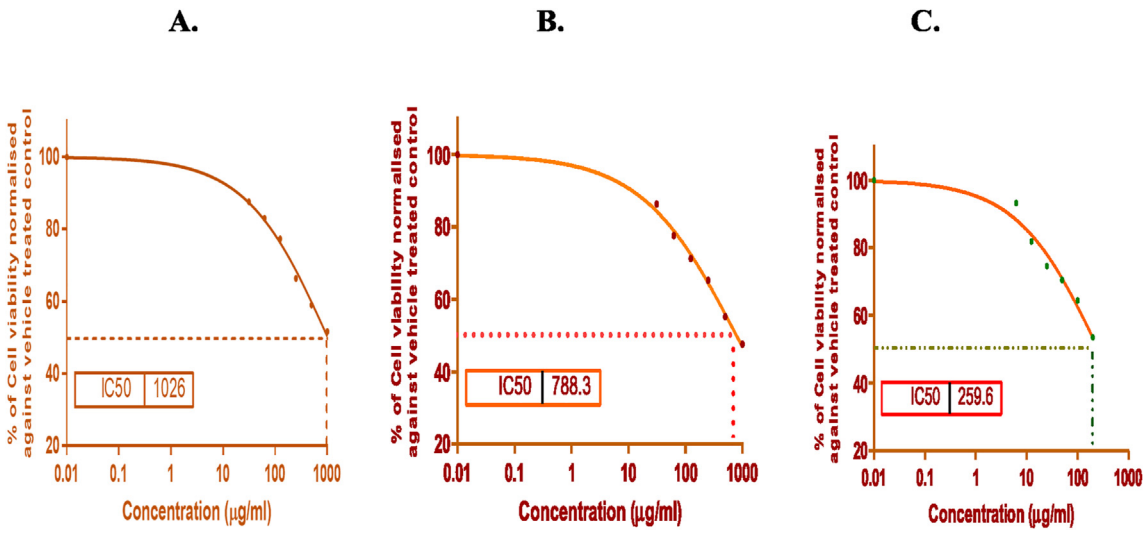

Figure 4. Evaluation of cell viability percentage of healthy human hepatic cells (THLE2) post-treatment A- SAF volatile compounds, B- SAF water extract compared with reference drug C– CIS using MTT assay.

studied plant extracts. In general, both water and volatile extracts exhibited a lower growth inhibitory activity against the Hep G-2 cell line than the reference drug, CIS (IC_{50} 20.71 µg/mL). According to the cytotoxicity scale established by Kuete et al. [39], the investigated SAF water-extract showed moderate cytotoxicity (50 μg/mL < IC50 < 200 μg/mL) while volatile oil has low cytotoxicity against cancer cell lines (200 μg/mL < IC50 < 1000 μg/mL). No cytotoxicity was detected in normal cell lines (IC_{50} > 400 μg/mL) for both extracts according to the same scale. This is consistent with the reports of Asif *et al.,* [38] who studied the cytotoxicity of star anise alcoholic extract and showed a moderate cytotoxic effect (IC_{50} 50.34 µg/ml) towards human colon cancer cell lines HCT-111.

According to Table 1, phenylpropanoids constitute *I. Verum* volatile oil's major components, whereas anethole, estragole, and foeniculin were the dominants. *I. Verum* volatile oil has recorded lower cytotoxicity could be due to its main constituents' low or no cytotoxic effect. The *trans*-Anethole, the main component of the oil, was examined for its cytotoxicity in RC-37 cells by Sharopov et al. [40], with IC_{50} value 100 mg L^{-1}. The incubation of hepatocytes with Anethole caused cell death with losses of cellular ATP and adenine nucleotide pools. Anethole exhibits apoptotic activity since it can deteriorate DNA. Thus anethole could be described as a moderate cytotoxic agent, according to Kuete et al., [39]. Meanwhile, Vannini [41] has assessed the cytotoxic, genotoxic, and apoptotic Estragole activities in the cell line Hep G-2 cell line and reported that Estragole was not cytotoxic, and it failed to induce DNA apoptosis or damage. To the best of our knowledge, nothing was reported concerning the cytotoxicity of Foeniculin. Concerning the cytotoxicity data reported above, no synergetic effect could be expected due to Foeniculin.

Flavonoids and phenolic compounds are well-known for their cytotoxic activity with promising results against tumors and cancer cell-line [42]. In agreement with the present investigation, most of the identified phenolics (Table 1) were reported to have cytotoxic activity, with cell proliferation in cell line tests. These compounds are included Chrysin, Kaempferol, Quercetin, Apigenin, and Gentisic acids, which have shown a high to moderate cytotoxic effect against cell-line tests like prostate PC-3, hepatocellular HepG2, acute T-lymphoblastic leukemia CEM, prostate DU-145, breast, and HeLa cervical cancer cells [43]. Such higher activity than the volatile oil identified reveals a significant difference in the recorded IC_{50} for both extracts.

The extract, which contains phenolic compounds can occur a balance and mitigate the oxidative stress impacts [44]. These impacts are forcing fungi to produce mycotoxin as a cell-defense system [35]. The previous investigations revealed the efficacy of plant phenolic extracts against fungal growth and mycotoxin production [13, 45]. The mechanism of volatiles and phenolics to reduce mycotoxin, depending on these components' function as antioxidants, helps the fungi cell mitigate oxidative stress during their growth [37]. Also, compounds with antioxidant activity may affect the genetic and/or enzymatic pathway of toxin production [46].

Concerning the investigation results, implicated extracts showed a potent inhibition effect for the growth of toxigenic fungi, with more efficacies for volatile extract (of solid and liquid media; Figure 2). This inhibition is associated with the presence of bioactive components [37, 45]. Several compounds, which are existed in volatile extracts sourced from agro-food materials, were reported to possess antimicrobial impacts against pathogens [47]. This impact was recorded clearly in the present investigation by the volatile extract, with better efficiency on pathogenic bacteria strains (Figure 1).

The antimicrobial action could occur due to individual volatile activity or their synergism effect [48, 49]. Synergistic impacts were reported to occur in both. between volatile oil components themselves, or volatiles and phenolic compounds [49]. Extract-content of bioactive components, especially phenolic and antioxidants assist the fungal cells to mitigate their suffering of oxidative stress, which is considered the main reason for mycotoxins' formation [35, 44]. This could illustrate the mechanism by which the water extract application in liquid fungal-growth media was accompanied by an efficient reduction for mycotoxin secretion (Table 3). In this regard, several suggested applications could be presented for the SAF against toxigenic fungi and their mycotoxins, where the efficacy may depend on the methodoly of application. The SAF could be applied to protect the stored grains by the application to spraying the storage areas, or directly on cereal grains. Also, it may use as an additive in food and beverage products for the protection against prospective cross-contamination.

4. Conclusion

The Star anise variation of the species between different studies could explain the reported changes in their bioactivity and therapeutic efficacy. Imported SAF samples of Vietnamese species were purchased from local markets to evaluate their bioactivity. The GC-MS results of the SAF volatile extract showed an absence of adulteration, while the HPLC results of water extract demonstrate the diversity in the phenolic content. Volatiles exhibited more inhibition against bacteria and fungi than the water extract. However, the SAF-water extract exhibited moderate cytotoxicity

against Hep G2 cells compared to the low-cytotoxic impact that was recorded by the volatile oil. Although the previous investigation pointed out the antifungal impact of volatile extract, the present study gave evidence for the anti-mycotoxigenic impact of water extract that recorded more efficacy to reduce mycotoxin secretion. Even though long handling and storage could decrease the agricultural commodities content of minor components, the SAF still manifested an efficacy against mycotoxin contamination using a simulated model. This recommended their application in food safety; including cereal protection at pre and post-harvest stages.

Declarations

Author contribution statement

Bassem Ahmed Sabry: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Amr Farouk: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Ahmed Noah Badr: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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